Identification Of Novel Distinct Autoantigen Clusters Reflecting The Heterogeneity Of Systemic Lupus Erythematosus

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Introduction

Diagnosis of systemic lupus erythematosus (SLE) is based on a combination of clinical findings and laboratory evidence such as anti-nuclear autoantibodies (ANA), anti-Smith and anti-double stranded DNA (dsDNA) antibodies. However, no biomarker individually displays sufficient performance to diagnose SLE, to predict the disease course or to allow the identification of patient sub-groups. This lack of specific biomarkers also affects clinical development of new SLE therapeutics.

Luminex bead-based antigen arrays were employed to characterize in-depth the autoantibody reactivity of SLE as a source to develop improved diagnostic and patient stratification tests for SLE.

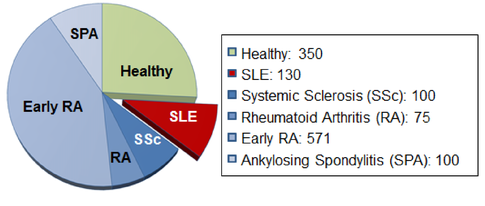


Fig. 1: Profiled patient samples

A large-scale screen of 5,885 recombinant human antigens was performed to profile SLE autoantigens. Serum samples from SLE patients were compared against healthy controls (HV) and samples from patients with other autoimmune diseases (AID) (Fig. 1). Autoantigens with significant autoantibody reactivity were selected and used to develop biomarker panels with improved sensitivity and specificity. Spearman's rank correlation of the top 50 SLE antigens was computed to identify novel autoantigen clusters.

Conclusions

- Comprehensive profiling of SLE sera enabled the in-depth characterization of the autoantigen repertoire of SLE patients.
- The combination of established and new antigens significantly increased the sensitivity to diagnose SLE.
- Based on their autoreactivity profile SLE sub-groups were revealed.
- Further studies are needed to link specific antigen clusters to clinical response profiles.

Methodology

The SeroTag® technology enables the discovery and validation of novel autoantigens using an automated multiplex platform (Fig. 2). The SeroTag® technology utilizes the bead-based Luminex xMAP technology which enables to measure the reactivity of autoantibodies to thousands of different antigens in one single serum sample. A crucial component of the discovery process is the unique warehouse of currently 6,500 human proteins expressed in E.*coli* (1). The Ni-NTA purified proteins are coupled to color-coded magnetic beads which enables the multiplex analysis of up to 500 different antigens. In this study SeroTag® was utilized in a non-hypothesis driven approach to identify novel SLE autoantigens.

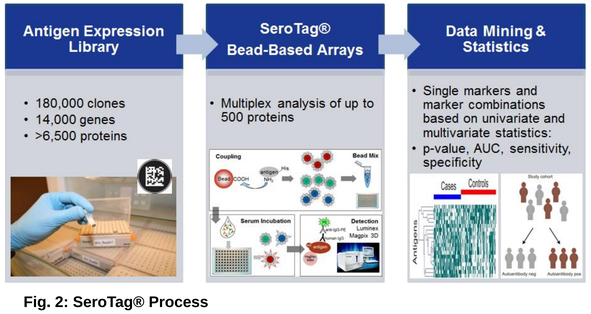


Fig. 2: SeroTag® Process

Study Design

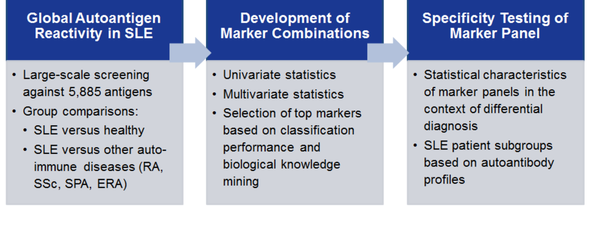


Fig. 3: Discovery and Performance Testing

Results

Antigen Selection Process

Statistical analysis was performed to distinguish SLE from HV and AID. Antigens with at least two-fold difference between the test groups (log2 ratio ≥ 1) and adjusted p-value <0.05 (log10<1.3) were selected. Fig. 4 shows the Vulcano plots in which the magnitude of the antigen reactivity in SLE sera relative to the control group is shown on the x-axis and the statistical significance on the y-axis.

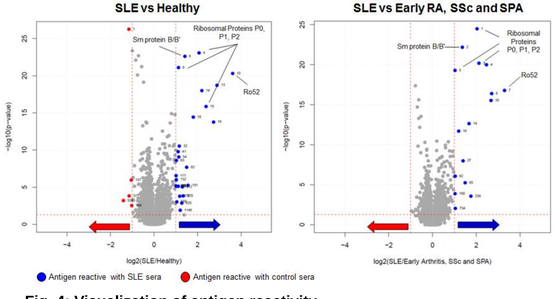
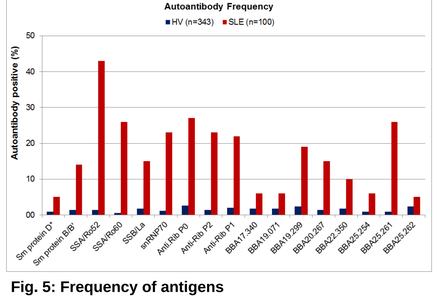


Fig. 4: Visualization of antigen reactivity

A cut-off (mean value plus 3 S.D. of HV) was defined and the frequency of antigens in SLE patients determined.



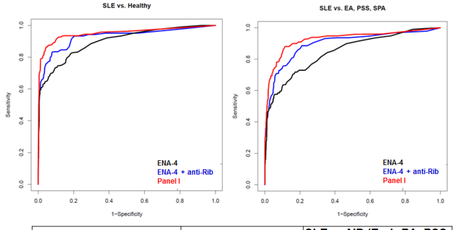
ENA-4	Clinical Diagnosis (%)
SM	8
SSA	37
SSB	10
U1 RNP	19

Fig. 5: Frequency of antigens

To address the heterogeneity of SLE 26 antigens were selected including a combination of established and new antigens including Rib proteins (2). The antigen panels with the best classification accuracy were defined using receiver operating characteristics (ROC) analysis.

Performance of Antigen Panels

Sequential addition and different combinations of antigens to a panel of known SLE antigens resulted in a stepwise improvement of the classification performance (Fig. 6).



SLE vs Healthy			Antigen Panels	SLE vs AID (Early RA, PSS, SPA)		
AUC	Sens.	Spec.		AUC	Sens.	Spec.
0.89	0.63	0.96	ENA-4	0.84	0.35	0.99
0.93	0.72	0.97	ENA-4 + anti-Rib	0.91	0.49	0.98
0.96	0.83	0.98	Combinatorial panel I	0.94	0.60	0.98

Fig. 6: Stepwise optimization of antigen panels

Antigen Clusters and Patient Subgroups

The ability of new antigens to segregate SLE from healthy controls was visualized using Powered Partial Least Squares discriminant analysis (PPLS-DA). The overlaid score (samples) and loading plots (antigens) show that the addition of new antigens significantly improves the separation of SLE samples from HV and reveals further sub-groups of SLE patients.

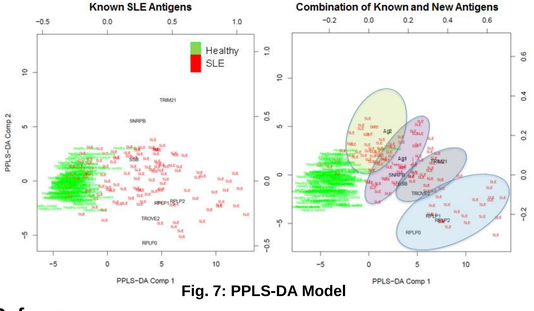


Fig. 7: PPLS-DA Model

References

1. Beyer NH, Lueking A, Kowald A, Frederiksen JL, Heegaard NH (2012). J Neuroimmunol. 242(1-2):26-32.
2. Barkhudarova F, Dähnrich C, Rosemann A, Schneider U, Stöcker W, Burmester GR, Egerer K, Schlumberger W, Hiepe F, Biesen R (2011). Arthritis Res Ther. 13(1):R20.